AMENDMENT

Please amend the application as indicated below. A version showing changes made is presented in Appendix A.

In the Specification:

Please amend the last paragraph of page ~~37~~ 38 to read:

Clones containing VL and VH sequences can be placed in an expression cassette incorporating a single-chain antibody construct including the VL and VH sequences separated by a linker. In one highly preferred procedure, the 5'-leader sequence is removed from VL and replaced with a sequence containing a Sal I site preceding residue 1 of the native protein. B1 Constant region residues from the 3'-end are replaced with a primer adding a sequence complementary to a sequence coding for a linker sequence (e.g., the 16-residue linker sequence ESGSVSSEELAFRSLD (SEQ ID NO: 5) (J. K. Batra et al., "Anti-TAc (FV)-PE40, a Single Chain

Please amend the last paragraph of page ~~59~~ 61 to read:

Generation of Surface-Linked scFv and 1-A^d expression constructs The generation and functional characteristics of surfaced-linked scFv constructs derived from the hamster mAbs 145-2C11 (anti-murineCD3ε ; Leo O et al., Proc Natl Acad Sci U S A. 1987. 84: 1374-8) and PV-1 (anti-murine CD28) and 5H7 (anti-humanClass1 MHC; Smith D, et al. J Immunol 1994. 153: B2 1054)are known in the art. Surface-expression for these proteins has been achieved using glycosylphosphatidylinositol (GPI) anchor motif. An anti-CTLA-4 scFv construct (4F10scFv) was generated from the hybridoma UC-10-4F10 which secretes a mAb with specific binding

affinity for murine CTLA-4. Total RNA prepared from hybridoma cells by the guanidinium isoiniocyanate CsCL method was used to synthesize cDNA with the First Strand cDNA kit (Novagen, Madison, WI) according to the manufacturer's instructions. Amplification of the V_H gene from cDNA was preformed with the following primers: Sense: 5' CGAATG**ATGCAT**CC(C/G)AGGTG(CA)AGCTG(C/G/A)(A/T)G(G/C)AGTC 3' (SEQ ID NO: 6) which incorporates an NsiI restriction site (in bold): Antisense: 5' GCAAAT**AAGCTT**TTGTTCGGCTGAGGAGACGGT(G/A)AC 3' (SEQ ID NO: 7) which incorporates a HindIII restriction site (in bold). Amplification of the V_L gene was performed with the primers: Sense 5' CGAATG**GACGTC**ATGATGACACAGTCTCC 3' (SEQ ID NO: 8) which incorporates an AatII restriction site (in bold): Antisense: 5' TATGAT**CCGCGG**AGGAACGTTT(T/G)ATTTCCAGCTTGGTCCC 3' (SEQ ID NO: 9) which incorporates a SacII restriction site (in bold). Cycling conditions were (93°C x 1 minute. 50° C x 1 minute. 72°C x 1 minute for 35 cycles using the DNA polymerase Pfu (Stratagene, La

Please amend page ~~60~~ 62 to read:

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Jolla, CA). The PCR products of the V_L and V_H genes were digested with the restriction endonucleases Aat II/SacII and Nsi I/ Hind III respectively. The digested PCR products were cloned into the temperature-inducible expression vector Genex in the orientation NH_2-4F10 VL-linker-4F10 VH-COOH. Surface-linked 4F10scFv (subsequently referred to as mem4F10scFv) was constructed by a tailed primer Polymerase Chain Reaction (PCR) strategy using 4F10scFv as a template. The primers used were: Sense: 5' GAGT**AAGCTT**ATGAGGACCCCTGCTCAGTTTCTTGGAATCTTGTTGCTCTGGTTTCC AGGTATCAAATGT*GACGTCATGATGACACAGTCTCC* 3' (SEQ ID NO: 10) which incorporates HindIII restriction site (in bold). A murine light chain leader peptide sequence (underlined) and nucleotide residues 4 to 26 of the sequence for 4F10scFv (in italics). Antisense: 5' AGCTT**CTTAAG**CTTCCGCTACCACTAGACACAGGGGCCAGTGGATAGACCGATGGG

GCTGTTGTTTTGGC*GGCTGAGGAGACGGTGACC* 3' (SEQ ID NO: 11) which incorporates an AflII restriction site (in bold) sequence encoding a flexible spacer peptide (underlined) and the final 19 residues of the sequence for 4F10scFv. Cycling conditions used to generate the modified 4F10scFv with these primers were: (94^0C x 1 minute). 1 cycle (94^0 x 30 seconds. 58^0 x 30 seconds. 72^0C x 1 minute). 5 cycles: (94^0 x 30 seconds 72^0C x 1 minute). 30 cycles: (72^0C x 10 minutes). 1 cycle. Amplification was carried out on a Geneamp 9600 thermal cycler (Perkins Elmer Corp., Norwalk. CT) using *Taq* DNA polymerase (Life Technologies). This resulted in a single product which was then digested with the restriction endonucleases HindIII and AflII (Life Technologies). Two surface-linkage strategies were used to generate a membrane bound form of the modified 4F10scFv (mem-4F10scFv). The first utilized a GPI anchor motif while the second utilized a cDNA fragment corresponding to the entire transmembrane domain and the first 34 amino acid residues of the cytoplasmic domain of the murine B7-1 (CD86) protein. The latter was generated by PCR using cDNA encoding murine B7-1 (CD80) as a template and with the following primers: Sense: 5' GAGCTG**CTTAAG***CAAGAACACACTTGTGCTC* 3' (SEQ ID NO: 12) which includes an AflII site (in bold) and the first 20 nucleotides of the B7-1 transmembrane domain)in italics). Antisense: 5' GTTCGC**TCTAGACT***AAAGGAAGACGGTCTGTTCAGC* 3' (SEQ ID NO: 13) which incorporates and Xbai site (in bold) and in-frame stop codon (underlined) and 22 nucleotide residues from the intracytoplasmic domain of B7-1 (in italics). Cycling conditions for generation of the surface-linkage domain were: (94°C X 1 minute), 1 cycle (94°C X 30 seconds, 57°C X 30

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Please amend the ~~first~~ paragraph of page ~~61~~ 63 to read:

seconds, 72°C X 1 minute), 35 cycles: (72°C X 10 minutes), 1 cycle. The modified 4F10scFv product was digested with the restriction endonucleases HindIII/AflII while the surface –linkage

motifs were digested with the enzyme pair AflII/Xbal. Final constructs were then assembled by simultaneous ligation of the digested 4F10scFv and the digested surface-linkage motifs into the HindIII and Xbal sites of the mammalian expression vector pCDBA3.1 (+) (Invitrogen Corp. Carlisbad, CA) at the HindIII and Xbal sites to generate mem4F10scFvpCDNA3.1(+). Complementary DNA sequences for the α and βchains of the murine Class II MHC protein I-A^d were generated by reverse transcription and polymerase chain reaction (RT-PCR). Total RNA was extracted from the BALB/c-derived B cell lymphoma cell line A20 using Trizol Reagent (Life Technologies) according to the manufacturers instruction. Reverse transcription with an oligo-dT primer was carried out using the Superscript II first strand cDNA synthesis kit (Life Technologies) by recommended protocol and was followed by PCR using the DNA polymerase mixture Elongase (Life Technologies). Primer for PCR amplification of the coding region of the two chains were derived from published sequence and were as follows: I-A^dα: Sense – 5' GAGCTG**AAGCTT***ATGCCGTGCAGAGCTCTGATTC TGG* 3' (SEQ ID NO: 14) (HindIII site in bold). Antisense – 5' GCCCGC**TCTAGA***TCATAAAGGCCCTGGGTGTCTGG* 3' (SEQ ID NO: 15) (Xbal site in bold): I-A^dβ Sense – 5' GAGCTG**AAGCTT***ATGGCTCTGCAGATCCCCAGC* 3' (SEQ ID NO: 16) Antisense – 5' GCCCGC**TCTAGA***TCACTGCAGGAGCCCTGCTGGAGG* 3' (SEQ ID NO: 17) . Conditions for PCR were: (94°C X 1 minute) 1 cycle: (94°C X 1 minute) 1 cycle: (94°C X 30 seconds, 57°C X 30 seconds, 68°C X 30 seconds) 35 cycles: (68°C X 10 minutes) 1 cycle. The resulting products were digested with the restriction enzymes HindIII and Xbal and ligated into the expression vectors pCDNA3.1(+)(I-A^dα) and pCDNA3.1(+)ZEO(I-A^dβ).

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In The Sequence Listing:

Please replace the Sequence Listing in its entirety with the enclosed substitute Sequence Listing, pages 1-7.

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